REMARKS

Disclosure:

The examiner objected to the disclosure because the terms "[sic]" and "[lacuna" appear and it was not clear what these terms mean. Applicants amend the specification in the places these terms appear without adding new matter.

As required, applicants insert a Brief Description of the Drawings section for Figure 1.

Claims 9 an 13 were objected to because of informalities. Applicants clarify the wording of these claims with proper amendments.

35 USC § 112 and § 101:

The examiner objected to recitation in claim 2 of the phrase "derive from" because the nature and number of derivative steps are not defined. Applicants believe "derive from" is not indefinite because as page 4, lines 24-29 of the disclosure shows "derive from" refers to the isolation of SEQ ID NO: or its homologs from *Ashbya gossypii*. Claim 2 is narrower than claim 1 because of this limitation.

Claims 14 and 15 were rejected as improper use claims. To overcome the rejection, applicants amend claims 14 and 15 into proper process claims comprising a definite step.

Claims 1-4 were rejected because the claims do not recite gene or amino acid sequence being isolated from or otherwise altered from its naturally-occurring form in nature in an Ashbya gossypii cell. Applicants refer the examiner to page 4, lines 24-29

of the specification and pointed out that the claimed genes are in fact isolated from organisms, preferably from *Ashbya gossypii*.

35 USC § 112, first paragraph:

Claim 10 was rejected under the written description requirement because of the use of the word "gene." Applicants replace "genes" with "nucleic acid sequence" as suggested by the examiner.

Claims 1-13 were rejected under § 112, first paragraph, because the claims encompass "homologs" of SEQ ID NO: 1 and the specification fails to describe common attributes or characteristics that identify members of the genus. The examiner also states that she found no genes having at least 80% homology to SEQ ID NO: 1 in the prior art.

Applicants respond by pointing out that the homologs of SEQ ID NO: 1 **are** disclosed in the specification on page 3 lines 26-44 and page 4 lines 1-7: "Homologs of the novel orotidine-5'-phosphate decarboxylase gene having the sequence SEQ ID NO: 1 mean, for example, allelic variants which have at least 80% homology at the derived amino-acid level... " The teachings in the specification about homologs of SEQ ID NO: 1 are clear. Also, the fact that homologs of SEQ ID NO: 1 could not be found in the prior art shows that the claims are novel over the prior art. Finally, as the person skilled in the art knows, a particular give sequence easily can be modified by genetic engineering to yield homologs without rendering the activity of the corresponding protein. Restricting the disclosed specific sequences SEQ ID NO: 1 would deprive the applicants of the merits of the present invention.

Please find enclosed a check for $110.00 for the one month extension of time.

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Respectfully submitted,
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VERSION WITH MARKINGS TO SHOW CHANGES MADE IN THE SPECIFICATION

Please amend page 2, lines 44 - 47 as follows:

However, genetic studies on riboflavin synthesis by Ashbya gossypii (vitamin B2 synthesis) have shown that the URA3 gene from Saccharomyces cerevisiae or the pyrF gene from Escherichia coli are [[sic]] not capable of complementation of

Please amend page 3, lines 4-15 as follows:

Attempts have therefore been made, because that [[sic]] gene from 5 Ashbya gossypii corresponding to the URA3 gene or pyrF gene is unknown, to clone it. Attempts at cloning the Ashbya gene by the methods described in the literature via, for example, hybridization with URA3 gene fragments or via degenerate oligonucleotides based on conserved amino-acid sequences of various orotidine-5'-phosphate decarboxylases and screening a cDNA library using these oligonucleotides and the PCR technique were unsuccessful (Bergkamp et al. Yeast, Vol. 9, 1993: 677 - 681, Piredda et al., Yeast, Vol .10, 1994: 1601- 1612, Benito et al., Gene, Vol. 116, 1992: 59- 67 and Diaz-Minguez et al., Mol. Gen. Genet., Vol. 224, 1990: 269 -278).

Please amend page 3, lines 22-28 as follows:

We have found that this object is achieved by the novel orotidine-5'-phosphate decarboxylase [[lacuna]] gene having the sequence SEQ ID NO: 1 or its homologs which have at least 80% homology with the sequence SEQ ID NO: 1.

Brief Description of Drawings

FIG. 1 is a pictorial depiction of the XhoI-SphI fragment of the construct ura 3::G418.

Please amend page 4, lines 19-22 as follows:

Derivatives also mean variants whose nucleotide sequence in the region from -1 to -200 in front of the start codon have [[sic]] been modified so as to alter, preferably increase, gene expression and/or protein expression.

Please amend page 4, lines 31-47 as follows

The novel gene construct means the URA3 gene sequences[[sic]] SEQ ID No.1 and its homologs which have been functionally linked to one or more regulatory signals, advantageously to increase gene expression. Examples of these regulatory sequences are sequences to which inducers or repressors bind and thus regulate the expression of the nucleic acid. In addition to these novel regulatory sequences, the natural regulation of these sequences in front of the actual structural genes can still be present and, where appropriate, have been genetically modified so that the natural regulation has been switched off and the expression of the genes has been increased. The gene construct can, however, also have a simpler structure, that is to say no additional regulatory signals have been inserted in front of the sequence SEQ ID No.1 or its homologs, and the natural promoter with its regulation has not been deleted. Instead,

the natural regulatory sequence has been mutated so that regulation no longer takes place, and gene expression is enhanced. The gene construct may

Please amend page 6, lines 34-41 as follows:

These regulatory sequences are intended to make specific expression of the genes and [of the [sic]] protein expression possible. This may mean, depending on the host organism, for example that the gene is expressed or overexpressed only after induction, or that it is immediately expressed and/or overexpressed.

Please amend page 7, lines 31-43 as follows:

The invention further relates to a process for inserting DNA into organisms, which comprises inserting into an organism, preferably a microorganism, which is deficient in an orotidine-5'-phosphate decarboxylase gene (= URA3 gene) a vector which comprises an intact URA3 gene having the sequence SEQ ID NO:1 or its homologs, advantageously together with further DNA, preferably with at least one other gene, and cultivating this organism on or in a culture medium which contains no uracil. Only these organisms which have acquired the vector are able to grow in this medium. A linear DNA is preferably used as vector in this process. The microorganisms preferably used in this process are fungi, especially of the family Metschnikowiaceae such as Eremothecium, Ashbya or Nematosprora [[sic]], particularly preferably microorganisms of the genus Ashbya.

Please amend page 8, lines 8-11 as follows:

The novel URA3 gene having the sequence SEQ ID NO: 1 or its homologs can advantageously be used as selection markers in the novel process. It is possible and preferred to insert genes using [this] these selection marker genes [[sic]] into Ashbya gossypii.

Please amend page 9, lines 7-18 as follows:

Genomic DNA from Ashbya gossypii ATCC10895 was prepared by the process described in WO97/03208. The genomic gene bank derived from this DNA was constructed in pRS314 and in YEp351 (Hill et al., Yeast, Vol. 2, 1986: 163-167) by the method described in Sambrook, J. et al. (1989) Molecular cloning: A laboratory manual, Cold Spring Harbor Laboratory Press or in [[lacuna]] F.M. et al. (1994) Current protocol in molecular biology, John Wiley and Sons. As can be inferred from, for example, WO97/03208, other plasmids, such as plasmids of the pRS series (Sikorski and Hieter, Genetics, 1989: 19-27) or cosmids such, as, for example, SuperCos1 (Stratagene, La Jolla , USA), are also suitable for producing the gene bank.

Please amend page 10, lines 40-41 as follows:

Degenerate olgonucleotides [[sic]] were synthesized on the basis of this information.

Please amend page 12, lines 39-46 as follows:

Disruption of a gene means destruction of the functionality of a gnomic copy of the gene either by (a) deleting part of the gene sequence or by (b) [of the[sic]] interrupting the gene by introducing a piece of foreign FAN into the gene or by (c) replacing part of the gene by foreign DNA. Any foreign DNA can be used, but it is preferably a gene which effects resistance to any

Please amend page 13, lines 4-12 as follows:

To disrupt the AgURA3 gene ob ashbya gossypii ATCC10895, the kananycin resistance gene from Tn903, which [[lacuna]] is under the control of the TEF promoter of Ashbya gossypii (see Yeast 10, pages 1793-1808, 1994 or WO92/00379), was used. The gene is flanked 5' and 3' by several cleavage sites for restriction endonucleases, so that it was possible to construct a cassette which [make[sic]] makes possible any desired constructions of gene disruptions using conventional methods of *in vitro* DNA manipulation.

Please amend page 13, lines 35-43 as follow:

Resulting G418-resistant clones were examined by conventional methods of PCT and Southern blot analysis (Sambrook, J. et al. (1989) Molecular cloning: A laboratory manual, Cold Spring Harbor Laboratory Press and in [[lacuna]] F.M. et al. (1994) Current protocols in molecular biology, John Wiley and Sons) to find whether the

gnomic copy of AgURA3 gene was in fact destroyed. Clones whose AgURA3 gene was destroyed are uracil-autotrophic and resistant to 1 mg/ml 5'-fluoroorotic acid (FOA).

VERSION WITH MARKINGS TO SHOW CHANGES MADE IN THE CLAIMS

Please amend claims 9,10 and 13-15 as follows:

9. (amended) A process for producing uracil-auxotrophic microorganisms, which comprises modifying an orotidine-5'-phosphate decarboxylase gene having the sequence SEQ ID NO: 1 or its homologs as claimed in claim 1 in such a way that the protein encoded by the gene is inactive, and [this modified gene is introduced] introducing this modified gene into the microorganisms and [integrated] integrating said gene by homologous recombination into the genome of the organisms, and subsequently selecting these microorganisms [are selected] for resistance to 5-fluoroorotic acid.

10. (amended) A process for inserting DNA into microorganisms, which comprises inserting a vector which comprises an intact orotidine-5'-phosphate decarboxylase gene having the sequence SEQ ID NO: 1 or its homologs as claimed in claim 1 together with at least one other [gene] nucleic acid sequence, into a microorganism which is deficient in orotidine-5'-phosphate decarboxylase [gene] nucleic acid sequence having the sequence SEQ ID NO: 1 or its homologs as claimed in claim 1 together with at least one other [gene] nucleic acid sequence, into a microorganism which is deficient in orotidine-5'-phosphate decarboxylase [genes] nucleic acid sequences, and cultivating this microorganism on or in a culture medium without uracil.

13. (amended) A process as claimed in claim 10, wherein at least one gene of riboflavin synthesis is inserted as [other] additional gene into the microorganism.

14. (amended) [The use of] A process for selecting cells, said process comprising the step of transforming cells with a gene sequence or its homologs as claimed in claim 1 [as selection marker].

15. (amended) [The use of] The process as claimed in claim 14[in] for *Ashbya gossypii*.